www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(9): 22-26

www.thepharmajournal.com
Received: 11-09-2015
Accepted: 14-10-2015

Shakhmaiev AE
National Technical University "Kharkiv Polytechnic Institute"

Gorbach TV
Kharkiv National Medical University

Bobritskaya LA
National University of Pharmacy, Kharkiv, Ukraine

Krasnopolsky Yu M
National Technical University "Kharkiv Polytechnic Institute"

Correspondence:
Shakhmaiev AE
National Technical University "Kharkiv Polytechnic Institute"

Preparation and cardioprotective effect analysis of liposomal coenzyme Q10

Shakhmaiev AE, Gorbach TV, Bobritskaya LA, Krasnopolsky YuM

Abstract
At the result of test experiments samples of LS form of Q10 were obtained. The optimum structure of the lipid, which allows the encapsulation to bilayer more than 80 % of the antioxidant was established and the technology of obtaining LS composition was proposed. When studying the pharmacological activity of the preparation antioxidant activity of water soluble form of LS form of Q10 on the models of myocardial infarction and ischemic heart disease was established.

Keywords: liposomal coenzyme Q10, antioxidant activity, ischemic heart disease

1. Introduction
One of the priority directions of developing modern pharmaceutical technologies is elaboration and creation of therapeutical systems of targeted action. To solve this problem nanosomal carriers of drug substances (polymeric [1, 2], pegylated particles [3], and liposomes (LS) [2-4]) are successfully used and are capable to increase targeting of action and provide the increasing bioavailability. LS forms comprising hydrophobic antioxidants: vitamin E, ubiquinone, curcumin and variety of other are actively developed [5, 6]. Previously we offered medicinal drugs based on LS compositions containing hydrophobic drug substances: "Lipoflavon" representing phosphatidylcholine liposomes with quercetin which is encapsulated bioflavonoid therein used in an injectable form and eye drops; "Lioliv" which has Antral hepatoprotector in its bilayer [3, 7-9]. Clinical researches which were carried out in Ukraine of LS drugs based on hydrophobic and hydrophilic substances demonstrated a high pharmacological activity in pulmonology, oncology, cardiology, nephrology, ophthalmology. The results of researches allowed to register drugs in Ukraine [3, 8].
Taking into consideration that the bioavailability of pharmaceutical drugs mainly depends on the solubility of the active substance, thus the aim of this research is to create a liposomal form of hydrophobic antioxidant for treatment ischemic heart disease (IHD).
Ubiquinone (ubidecarenone, coenzyme Q10) is a natural substance, vitamin-like coenzyme participating in the transfer of electrons in the transport chain of redox processes, in the process of energy exchange, in oxidative phosphorylation reaction of the cell mitochondrial respiratory chain. It participates in the process of cellular respiration increasing the synthesis of ATP. It gives clinically significant antioxidant effect. It protects lipids of cell membranes from lipid peroxidation processes. It reduces the area of myocardial injury under ischemia. Q10 prevents elongation of the QT interval, improves physical exercise tolerance. Due to the endogenous synthesis 100% body need for coenzyme Q10 is satisfied only up to 20 years of age, since then its synthesis is significantly reduced.
Q10 exhibits: antioxidant, antihypoxic, cardioprotective, cardiotonic, immunostimulation effect; transfers hydrogen ions, it is a component of the respiratory chain. Q10 is recommended to use in IHD, including myocardial infarction (acute, subacute), rehabilitation therapy, heart failure, with an increasing athletes loading [10, 11].

2. Materials and Methods
Used substances. The natural phospholipids (PL) such as phosphatidylcholine (PC) from egg yolks of company «Lipoid, 'LIPOID E PC S'» (96%), dipalmitoylphosphatidylglycerol (DPPG) (99%) manufactured by «Lipoid» were used. Q10 (98, 0%) manufactured by «Sinoway industrial Co» was used.

Experimental

Preparation of liposomes. LS were obtained due to methods [9, 11-13]. As the components of the PL film have to be uniformly distributed, Q10 was dissolved in an organic solvent comprising the PL in certain concentrations. The solvent was completely removed with a rotary vacuum evaporator at the temperature of 38-42 °C, and then the residual quantity of solvent was evaporated under nitrogen. The resulting thin lipid film was hydrated in an aqueous solution or a lactose solution with a water bath at the temperature of 40-42 °C to obtain a homogeneous suspension. Homogenization was carried out by means of extrusion with apparatus «Microfluidics-110Y» at previously established rates of pressure [9, 15]. Then was provided sterilization filtration through filters with a pore diameter of 0.8/0.22 μm. The emulsion was filled into flasks and lyophilization followed by sealing in an inert gas atmosphere. As cryoprotectant lactose (or trehalose) at different PL: cryoprotectant ratios (1:1, 1:2, 1:3, 1:4) was used. Resulting sterile lyophilized samples of LS ubiquinone were dissolved in sterile water. Resulting samples represented yellow homogeneous emulsion.

Pharmacological research

Research of LS form of Q10 was performed by two models of myocardial infarction (MI) and IHD. Wistar rats (about 3 months of age) weighting 150-180 grams were used underway [16, 17].

1. Simulation of MI was carried out by single intraperitoneal injection of 0.25 ml of 0.18% adrenaline solution. LS samples were injected: a) with a single dose 30 minutes before the start of the experiment at a dose of 10 mg/kg, intravenously; b) daily for 5 days after the simulation at a dose 10 mg/kg, intravenously. Lipid peroxidation (LPO) was evaluated by the content of thiobarbituric acid (TBA) representing active products which are malondialdehyde (MDA) and diene conjugates (DC). Activity of LS was evaluated by the total antioxidant activity (TAA). Also the content of isoprostane-8 representing a marker of oxidative stress was determined.
2. Simulation of IHD was performed by the method described by Haman L.V.: 0.1 ml of 0,1% adrenalin solution and 1 ml of 2.5% suspension of hydrocortisone were injected subcutaneously daily for 7 days. Samples were injected daily for 5 days after the simulation at dose 10 mg/kg intravenously.

Analytical research

Determination of particle size of LS was performed by nanosizer "Shimadzu SALD-1701" by photon correlation spectroscopy. Particle size was measured using a semiconductor laser with a wavelength of 375 nm and a temperature of 30 °C.

For the quantitative determination of impurities and content of Q10 in the test samples was used HPLC in the isocratic mode on chromatograph "Agilent 1100", chromatographic column 150 x 4.6 mm size filled with sorbent "Zorbax SB-C18" with a particle size of 3.5 μm ("Hewlett Packard"), mobile phase: methanol: absolute ethanol (65:35); mobile phase rate is 1 ml/min; detection at wavelength 275 nm; detection time is 20 minutes; column temperature is 35 °C. Volume of test sample is 25 μl. The average retention time of Q10 was 7.8 ± 0.1 min., which is conformed to the retention time of the standard sample Q10. Retention time of PL was not excess of 2 minutes. Impurities of Q10 was not more than 0.5%, which is correspond to the data specified by the manufacturer. Suitability of chromatography system was confirmed according to the State Pharmacopoeia of Ukraine (SPU).

Chromatography in thin-layer of silica gel was performed on plates "Silufol" in chloroform: methanol: water (65: 25: 4). For identification of PL structure standard samples of PL from "Sigma" company were used.

Oxidation index (OI) was determined by UV–spectroscopy at two wavelengths: 233 nm / 215 nm [18]. For identify the structure of the PL and fatty acid methyl esters standard samples of "Sigma" company were used. The ethanol content in the obtained samples was determined by method gas-liquid chromatography (GLC) by chromatograph HP-6880 according to SPU. Determination of fatty acid methyl esters was carried out by gas chromatography "Selmihrom-1" with a flame ionization detector (a stainless steel column with length of 2.5 m and an inner diameter of 4 mm, filled with an inerton stationary phase, which was treated with 10% diethylene glycol succinate). Methylation of fatty acids was carried out in a mixture of chloroform: methanol: sulfate acid (100:100:1).

The determination of statistical significance of the experimental results were conducted according to SPU 1.1.

3. Results and Discussion

To evaluate effectiveness of carrying out technological process method of quantitative measurement of Q10 in LS and evaluation of stability of Q10 in all technological operations was used. The presence of impurities in the primary product and, under technological process during receiving of LS form of Q10 was determined. With research of model mixture (ethanol and chloroform solutions) by HPLC it was revealed that the presence in mixtures of PL does not influence on results of chromatography.

In previous experiments we have found that the optimum content in LS is about 10% of Q10 from PL content. Increase of Q10 content in the samples more than 10.0% resulted in incomplete encapsulation into LS [9]. It was carried out study of encapsulation of Q10 in LS emulsion from various types of PL at a ratio of PL:Q10 - 9:1. [19]

As forming of LS occurs only in the liquid-crystalline state, the mixture of saturated PL must be heated to the phase transition temperature before being converted to LS. The phase transition temperature for the PL derived from natural sources (soybeans, egg lecithin, brain), with unsaturated fatty acids thereof is significantly lower than room temperature (from -10 °C to 35 °C) depending on the nature of the PL. It gives the advantage that for the formation of LS one can work at lower temperatures, that is comfortable for active pharmaceutical substances, which are sensitive to high temperatures. Hydrogenated or synthetic PL has the phase transition temperature significantly higher than 50-60 °C, and thus manufacturing of LS will take place at temperature of about 70 °C [3].

Preparation of LS was carried out at higher temperatures than the phase transition temperature of the lipids. Increase of the temperature to 50-70 °C resulted in the appearance of impurities in the LS comprising Q10. The degree of encapsulation of Q10 in LS is essentially the same. The OI was increased slightly to 0.22 (primary was 0.19). This may be due to the antioxidant properties of Q10. Given the appearance of impurities Q10 at high temperatures, in further experiments and during development of the drug we were used PC of egg yolk.

Study of the various stages of preparation technology of LS form of Q10 were performed in the following experiments. The first stage of technology is preparation of the lipid film.

Considering the fact that Q10 is essentially insoluble in water, it is dissolved in ethanol and mixed with ethanol solution of PL. The Q10:PL ratio from 1:10 to 3:10 was used during the operation. As PL egg PC and DPPG were used. The solution was concentrated under vacuum at temperature of 37-42 °C. Then the resulting film of PL with Q10 substance was emulsified in an aqueous solution to produce multilamellar vesicles. The temperature during the process of resuspension should be above the phase transition temperature of PL. Obtaining the vesicles the influence of several factors: temperature (35-45 °C), pH (6.0-7.5), the ionic strength of the solution, 0.9% sodium chloride, buffer mixtures of different molar concentration, lipid concentration and the ratio of PL: Q10 were studied. The size of the resulting vesicles is also defined by the intensity and time of stirring. For prevention of the oxidation processes of PL to LS, the resulting emulsion is saturated with nitrogen. As a result of the studies it was found that the optimal conditions of preparation a uniformly stable emulsion are the ratio of Q10 (1-2):PC (8-10):DPPG (1-2) at a temperature of 37-43 °C, the emulsion pH 6.5–7.0. By the method HPLC it was revealed that during the stage of PL film preparation Q10 was stable, because there are no impurities after processing at a temperature of 35-40 °C and emulsion pH 6.5-7.0.

LS were prepared by extrusion: the pressure was 800-1000 atm and a temperature 37-43 °C. This mode allows to get the LS having standard formulation, the bulk of which are represented by particles having a size of 120-160 nm (80-90% of all LS) before lyophilization and (140-190 nm) after lyophilization. LS larger than 220 nm were absent.

By HPLC method it was revealed that in the PL emulsion homogenization stage Q10 is not damaged, as evidenced by the absence of impurities after processing at a temperature of 37-43 °C and a pressure of 800-1000 atm.

OI of LS obtained by this method do not exceed 0.2-0.25 (OI of initial PC is not more than 0.2). PL stability and absence of new impurities, such as lysophosphatidylcholine, were established by TLC method. Using of obtained LS as medicinal product for intravenous administration is possible due to the nanoscale, low degree of oxidation and absence of degradation products of PL and Q10. The introduction of negatively charged PL (DPPG) into LS formulation leads to increase of Q10 incorporation in LS.

We have found that increasing of the number of cycles or the pressure value leads to instability of LS, increase size or fusion thereof. Control of homogenization process was evaluated by size of LS, and with the increase of this parameter process was finished. LS form of Q10 was obtained at a pressure of 800 atm, and the number of cycles was not more than 5. The tests to study the influence of LS size and its charge on the incorporation degree of Q10 were carried out. It was found that the stability of LS and Q10 incorporation into their structure influences the ionic strength, pH value, temperature of the technology process, charge and size of LS. Furthermore, independent value has stability of the drug itself, which is Q10, incorporated in LS.

Degree of encapsulating Q10 in PL bilayer using the ratios Q10:PC (1:9), and the ratio Q10:PC (2:8) is the same, that indicates the maximum saturation of LS membrane by antioxidant. However, we have found that the encapsulation of DPPG in LS permits to increase encapsulation of Q10 in LS by 30%. The optimal number of cycles, allowing to obtain LS at the nanoscale is not more than 5. Thus nanoparticles with a size of up to 220 nm nanoparticles with a size of up to 220 nm were obtained, allowing to perform a sterilization filtration through 0.22 μm membranes.

While increase of the number of cycles leads to the decreasing of the size of LS, however it decreases Q10 encapsulation to the membrane. The temperature of the homogenization of the emulsion 38-43 °C at which the OI was practically identical to the OI of the initial mixture of PL was established. Increase of the temperature to 45-50 °C leads to increase of the OI.

The presence in LS of a negatively charged PL (DPPG) permits, firstly, to increase Q10 encapsulation into membrane of LS, and secondly, increases the stability of the LS emulsion. Furthermore, DPPG improves the ability to filtrate emulsion through the membranes with pore size of 0.22 microns.

After obtaining Q10 nanoemulsion and setting the main extrusion parameters it was necessary to perform study of the stability of the Q10 substance and PC components during extrusion. The study of the amount of main substance and amount of impurities of each component of the sample was carried out. Q10 was controlled by HPLC method, phospholipids were controlled by TLC method.

It was found stability of all LS components during the technological process. Moreover, the components are stable, even when using modes that significantly exceed the requirements for obtaining LS form of Q10 (7 cycles at 1200 atm).

Cryoprotectant (CP) for the LS preparation should be determined experimentally. The best results for LS preparations were obtained when using carbohydrate compounds, such as sugars. A significant factor in obtaining of LS is the point of introduction of CP into nanoemulsion formulation. Paramount importance has chemical structure of CP, its concentration, the form of introduction and the point of introduction during the process of homogenization, i.e. at which the size of LS (at which cycle) to carry out its addition.

Number of experiments were carried out in order to study the influence of the introduction of sugars into the PL emulsion for the degree of Q10 encapsulation into the lipid membrane. We used three sugars: lactose, trehalose, sucrose, which are commonly used in the nanostructures lyophilization. Sugars were added at a concentration of 20 to 80 mg/ml.

Table 1: Influence of various sugars on Q10 encapsulation in LS at different stages of process

Name of CP	Introduction of sugar during rehydration of the film, mg/ml	Q10 encapsulation, %	Introduction of sugar during homogenization, mg/ml	Q10 encapsulation, %
Sucrose	20 to 80	52.4±4.7	20 to 80	61.6± 2.4*
Trehalose	20 to 80	86±3,1*	20 to 80	83.0±1.4*
Lactose	20 to 80	84.7± 1.6*	20 to 80	83.6±1.5*

P < 0,01 the reliability between Q10 encapsulation when using trehalose and lactose compared to use of sucrose.
n=5 (in all groups of experiments)

Sugars were introduced in the concentrated sterile solution form in the first stage during hydration of the lipid film by an aqueous solvent, and between 3 and 4 cycles. It has been found that the introduction of the test sugars in hydration step leads to a reduction of the Q10 encapsulation into structure of LS. Addition of sugars solutions (lactose, trehalose) during the

homogenization process leads to Q10 encapsulation into LS up to 65-70%.Thus, the carbohydrate protectors (e.g., lactose or trehalose) in the first stage may lead to an undesirable change in the size of LS and reduce encapsulation into them of the medicinal active pharmacological substance. At the same time, special attention should be paid to the achievement of the required size of LS and the exclusion aggregation thereof. Taken into consideration that the sucrose leads to decrease of Q10 encapsulation in LS further we used lactose as CP (as trehalose opposite to lactose is not described in pharmacopeias of Ukraine, Europe, USA, and others.).The main criterion for selection of CP is maintaining stability of sizes during lyophilization of LS membranes. For determining the optimal concentration of lactose in the preparation, addition of a solution of lactose in the LS emulsion formulation was performed between 3 and 4 cycles of homogenization to the final concentration of sugar of 20, 40 and 80 mg/ml (Table 2).

Table 2: Influence of lactose concentration on the stability of the LS nanosized (**)

Concentration of lactose, mg/ml	Q_{10} encapsulation in LS before lyophilization, %	LS size before lyophilization, nm	Q_{10} encapsulation in LS after lyophilization, %	LS size after lyophilization, nm
20.0	70.3±2.3	153.6±28.4	68.6±7.15	210±40.6
40.0	73.8±2.6	151.2± 30.1	69.3±1.87	195.6±23.7
80.0	80.4±2.7	151.4±26.7	84.2±1.85	162±28.6*

* P<0,01 the reliability between LS size after lyophilization depend on lactose content
** results obtained in samples without sterile filtration
n = 5 (in all groups ups of experiment)

As you can see from Table 2, use of different concentrations of lactose leads to obtaining of similar LS sizes before lyophilization. At the same time, after the performing of preparations lyophilization process the maintaining of nanoparticle sizes in the nanoscale range is observed only at concentrations of CP, lactose in a final concentration of 80 mg/ml.

As take into consideration that Q10 is stable in a neutral environment we were introduced in structure of LS potassium dihydrogen phosphate and disodium hydrogen phosphate (pH was 6.6 to 7.4). The resulting LS form of Q10 is a nanoemulsion with a particle size of 140-190 nm. Encapsulation of Q10 in LS after sterilizing filtration of the emulsion and separation of free Q10 was not less than 99%. After lyophilization due to lactose the samples of LS maintain nanosized and contents of encapsulated Q10.

Pharmacological study of LS form of Q10.

In the following group of experiments pharmacological activity of LS form Q10 study was carried out (Table 3-7).

Table 3: The results of test of antioxidant activity of LS form of Q10 on the model of MI (blood serum)

Group	DC, µmol/l	MDA, µmol/l	Isoprostane-8, ng/ml	TAA (%)
Control, n=8	52.34±2.02	2.45±0.21	5.42±0.34	57.48±3.11
MI, n=8	92.14±6.34[1]	6.59±0.32[1]	12.58±1.02[1]	45.38±2.18[1]
Administration of preparation, 5 days; n=8	60.48±3.11 P<0.05 P_1<0.001	3.00±0.25 P>0.05 P_1<0.001	5.00±0.37 P>0.05 P_1<0.001	60.49±3.02 P>0.05 P_1<0.001
Single administration; n=8	81.25±3.11 P_1<0.01	5.42±0.32 P_1<0.02	7.48±0.66 P_1<0.01	59.32±2.11 P_1<0.02

[1] p<0.001; P is reliability of differences with control; P_1 is reliability of differences with a MI; TAA is expressed in percentage (%) of inhibition; 50% of inhibition is 1 unit of activity.

Table 4: The results of test of antioxidant activity of LS form of Q10 on the model of MI (cardiac muscle)

Group	DC, µmol/l	MDA, µmol/l	TAA (%)
Control, n=8	1.72±0.11	0.325±0.008	63.28±3.12
MI, n=8	4.79±0.21[1]	0.589±0.031[1]	57.48±2.34[1]
Administration of preparation, 5 days; n=8	2.05±0.18 P>0.05 P_1<0.01	0.402±0.021 P<0.05 P_1<0.01	76.49±2.64 P<0.05 P_1<0.01
Single administration; n=8	3.89±0.28 P<0.01 P_1<0.02	0.419±0.021 P<0.02 P_1<0.01	69.45±3.64 P<0.05 P_1<0.01

[1] p<0.001; P is reliability of differences with control; P_1 is reliability of differences with a MI;

Table 5: The results of test of antioxidant activity of LS form of Q10 on the model IHD (blood serum)

Group	DC, µmol/l	MDA, µmol/l	Isoprostane-8, ng/ml	TAA (%)
Control, n=8	52.34±2.02	2.45±0.21	5.42±0.34	57.48±3.11
IHD, n=8	76.48±3.05 P<0.001	4.93±0.31 P<0.001	9.78±0.55 P<0.001	44.85±2.11 P<0.001
Administration of preparation, 5 days; n=8	61.22±4.17 P<0.01 P_1<0.001	3.37±0.28 P<0.01 P_1<0.02	6.00±0.34 P>0.05 P_1<0.01	52.88±1.84 P<0.05 P_1<0.01

[1] p<0.001; P is reliability of differences with control; P_1 is reliability of differences with a IHD;

Table 6: The results of test of antioxidant activity of LS form of Q10 on the model IHD (cardiac muscle)

Group	DC, µmol/l	MDA, µmol/l	TAA (%)
Control, n=8	1.78±0.12	0.325±0.008	63.23±3.11
IHD, n=8	3.47±0.18 P<0.001	0.489±0.007 P<0.01	53.23±2.77 P<0.01
Administration of preparation, 5 days, n=8	2.03±0.11 P>0.05 P_1<0.01	0.332±0.008 P>0.05 P_1<0.01	65.33±4.13 P>0.05 P_1<0.01

1 – p<0.001; P - reliability of differences with control; P_1 - reliability of differences with a IHD;

The data presented in Tables 3-6 demonstrated antioxidant activity of LS form of Q10, which manifests in lowering peroxidation products in model experiments when administrated LS samples containing Q10 and recovery of these compounds to their content in the control animals.

4. Conclusions.

At the result of test experiments samples of LS form of Q10 were obtained. The optimum structure of the lipid, which allows the encapsulation to bilayer more than 80 % of the antioxidant was established and the technology of obtaining LS composition was proposed. The incorporation to LS structure of a negatively charged DPPG allowed to increase the amount of incorporated into the bilayer Q10.

When studying the pharmacological activity of the preparation antioxidant activity of water soluble form of LS form of Q10 on the models of myocardial infarction and ischemic heart disease was established.

5. References

1. Deffeys K, Deffeys S. Amazing nanostructures. Binom, Moscow, 2011, 206.
2. Shvets VI, Kaplun AP, Krasnopolsky YM. From Liposomes of the 1970s to 21st Century Nanobiotechnology. Nanotechnologies in Russia 2008; 3(11-12):643-655.
3. Krasnopolsky YM, Dudnichenko AC, Shvec VI. Pharmaceutical biotechnology: Bionanotechnology in pharmacy and medicine. Publishing Center NTU "KPI", Kharkov, 2011, 227.
4. Grigoryeva GS, Stefanov OV, Krasnopolsky YM. Physical-chemical grounds of the membrane tropic factors in mechanism of the liposomal medicines action. International liposome society: Progress in drug and vaccine delivery, London, 2005, 50-54.
5. Patra D, Al-Khonry E, Ahmadich D. Effect of curcumin in liposome: curcumin as a molecular probe for monitoring interaction of ionic liquids with 1, 2 dipalmitoyl – sn-glycerol-3-phosphocholine liposome. Photochem. Photobiol. 2012; 88(2):317-327.
6. Xia Fei, Jin Heyang, Zhao Yaping, Cuo Xingiu. Preparation of coenzyme Q-10 liposomes using supercritical anti-solvent technique. J of Microencapsulation 2012; 29(1):21-29.
7. Antipova SV, Shepilov AV, Ryabtseva OD. Experience of Lipoflavon application to prevention development of cardiac complications in patients with operable breast cancer treated with anthracyclines. Problems of modern medical science and education 2009; 2:44-46.
8. Compendium. Medications. Morion, Kiev 2012, 1(2):1025,
9. Shakhmaiev AE, Krasnopolsky YM, Volchik IV. Technological principles of producing liposomal drugs. Ukr. Biopharm. Journal 2012; 21(4):4-11.
10. Verma DD, Hartner WC, Thakkar V. Protective effect of Coenzyme Q10 loaded liposomes on the Myocardium in Rabbits with an Acute Experimental Myocardial infarction. Pharmaceutical Research 2007; 24(11):2131-2138.
11. Wen-Chuan Lee, Tung-Hu Tsai. Preparation and characterization of liposomal coenzyme Q-10 for in vivo topical application. Pharmaceutics 2010; 395(1-2):78-83.
12. Krasnopolsky YM, Stepanov AE, Shvets VI. The lipid technology platform to create new formulations and transport of pharmaceutical substances. Biopharm. Journal. 2011; 3(2):10-18.
13. Shakhmaiev AE, Volchik IV, Krasnopolsky YM. Liposomal nanoparticles as carriers of drugs. Farmacom 2011; 3:88-95.
14. Shakhmaiev AE, Volchik IV, Krasnopolsky YM. Study of the impact of technological parameters on the properties of liposomal nanoparticles. Farmacom 2012; 1-2:82-87.
15. Krasnopolsky YM, Stepanov AE, Shvets VI. Technological aspects of obtaining liposomal drugs under GMP. Biopharm. Journal. 2009; 1(3):18-29.
16. Haman LV, Kononenko MI, Tyubka TY. Features of morphological and functional cardiac ultrastructure in experimental myocardial ischemia. Ukr. Biopharm. Journal. 2011; 10(5):16-20.
17. Preclinical studies of drugs: Methodic recommendations. Ed. By corresponding member Academy of Medical Sciences of Ukraine A.V. Stefanov. Avicenna, Kiev, 2002, 567.
18. Kates M. Techniques of lipidology. Elsevier, Amsterdam, 1986, 464.
19. Shakhmaiev AE, Gorbach TV, Volchik IV, Krasnopolsky YM. Creation of a liposomal form of a hydrophobic antioxidant ubiquinone. Farmacom 2014; 3:27-35.